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(21) International Application Number: PCT/AU92/00164 (22) International Filing Date: 13 April 1992 (13.04.92) (30) Priority data: PK 5664 16 April 1991 (16.04.91) AU (71) Applicant (for all designated States except US): COMMON-WEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; 14 Limestone Avenue, Campbell, ACT 2601 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only) : WARD, Kevin, Alan [AU/AU]; 28 Woodbury Street, Wordford, NSW 2778 (AU). NANCARROW, Colin, Douglas [AU/AU]; 47 Chelmsford Avenue, Willoughby, NSW 2068 (AU). BROWNLEE, Alan, George [AU/AU]; 8/1 Pennant Street, Castle Hill, NSW 2154 (AU).		(74) Agent: F.B. RICE & CO; 28A Montague Street, Balmain, NSW 2041 (AU). (81) Designated States: AT (European patent), AU, BE (European patent), BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>
(54) Title: GENE EXPRESSION CASSETTE CONTAINING NON-CODING SEQUENCE OF GROWTH HORMONE GENE (57) Abstract <p>The present invention provides a genetic expression cassette for use in obtaining expression of a cDNA sequence in animal cells. The expression cassette comprises an inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene or a portion thereof. The cDNA sequence is inserted between the inducible promoter and the exon 5 of the growth hormone genes.</p>		

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GENE EXPRESSION CASSETTE CONTAINING NON-CODING SEQUENCE OF GROWTH HORMONE GENE

FIELD OF THE INVENTION

The present invention relates to a gene expression cassette which enables expression of cDNA sequences in animal cells. The expression cassette of the present invention is particularly useful in achieving high-level expression of bacterial and/or plant genes in animal cells.

BACKGROUND OF THE INVENTION

It is now possible to transfer unique pieces of DNA between organisms in such a way that the transferred material becomes a functional part of the genetic information of the recipient organisms. The animals that are produced by this technique are termed "transgenic". One application of this technology is to transfer biochemical pathways from bacteria to domestic animals in order to increase animal productivity. One difficulty which is frequently encountered in efforts to produce such transgenic animals is the lack, or very low levels of expression of the transferred DNA sequences.

The present inventors have developed a genetic expression cassette which provides information for the expression of heterologous genes, in particular bacterial genes, in mammalian cells and in several tissues of transgenic animals, at levels that provide ready detection of the encoded polypeptides.

The expression cassette consists of two components:- a regulatory element and a non-coding sequence from the growth hormone gene.

SUMMARY OF THE PRESENT INVENTION

Accordingly, in a first aspect the present invention consists in a genetic expression cassette for use in obtaining expression of a cDNA sequence in animal cells, the cassette comprising an inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene or a portion thereof, the cDNA sequence being positioned

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between the inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene.

In a preferred embodiment of the present invention the inducible promoter is the immediate upstream nucleotide sequence of the sheep metallothionein-Ia gene.

The expression cassette of the present invention provides a means for the expression of a wide range of genes in transgenic animals, including the coding sequences of bacterial enzymes, plant chitinases, insecticidal scorpion venom toxin and the insecticidal protein of the bacteria Bacillus thuringiensis. In a preferred embodiment of the present invention the cDNA sequence is selected from the group consisting of cysE, cysK, aceA and aceB genes of Escherichia coli and the coding sequences of plant chitinases.

In yet a further preferred embodiment of the present invention the genetic expression cassette has a sequence substantially as shown in Figure 1.

The expression cassette of the present invention is useful in obtaining high levels of expression of cDNA sequences in animal cells. Accordingly, in a second aspect the present invention consists in a non-human animal including the genetic expression cassette of the first aspect of the present invention.

In a preferred embodiment of this aspect the animal is ovine or bovine.

DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples and figures in which:-

Figure 1 shows the nucleotide sequence of the expression cassette of the present invention;

Figure 2 shows the sequence of MTCE10;

Figure 3 shows the sequence of MTCK7;

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Figure 4 shows the sequence of MTCEK1;

Figure 5 shows the sequence of MTAceA2;

Figure 6 shows the sequence of MTAceB2;

Figure 7 shows the sequence of MTAceAB11; and

5 Figure 8 shows levels of radiolabelled cysteine in transgenic mice containing MTCEK1 (——) and in control mice (---). The arrow shows the position of cysteic acid.

Initially, a number of gene arrangements for
10 expression of the cysK gene in murine L-cells were trialled. The trialled constructs were as follows:-

pMTCK7 - sheep metallothionein-Ia gene promoter - cysK - exon 5 of sheep growth hormone.

pMTCK8 - sheep metallothionein-Ia promoter - exon 1
15 sheep growth hormone - cysK - exon 5 sheep growth hormone.

pMTCK11 - sheep metallothionein-Ia promoter - cysK - whole sheep growth hormone.

pMTCK12 - sheep metallothionein-Ia - exon 1 sheep
20 growth hormone - cysK - exons 2, 3, 4 and 5 sheep growth hormone.

The constructs were transfected into murine L-cells and the O-acetylserine sulfhydrylase activity of the transfected cells measured. The results obtained are set out in Table 1.

25

TABLE 1

O-Acetylserine Sulfhydrylase Activity in Transfected Murine L-Cells Using Various cysK Genes

<u>Gene</u>	<u>Enzyme Activity</u>
	(nMoles cysteine produced/mg protein/30 min)
pMTCK7	1350 ± 24
pMTCK8	510 ± 13
pMTCK11	162 ± 17
pMTCK12	159 ± 6

35 (values represent the means of two determinations)

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As can be seen from these results exon 5 of the growth hormone gene of sheep is required for optimum expression of genes inserted into the cassette. Other combinations which comprise larger portions of the sheep growth hormone gene are less effective in providing expression.

Two examples of the function of the expression cassette are shown as follows:

1. Expression of the cysE and cysK genes of E. coli in transgenic animals

In order to provide a pathway for the biosynthesis of the amino acid cysteine, the coding sequences for the bacterial enzymes serine transacetylase and O-acetylserine sulfhydrylase have been inserted into the expression cassette.

Three genes are described. Genes 1 and 2 each encode single bacterial proteins, gene 1 encoding the protein serine transacetylase and gene 2 encoding the protein O-acetylserine sulfhydrylase. Gene 3 is a compound gene constructed from gene 1 and gene 2, and encodes both the serine transacetylase protein and the O-acetylserine sulfhydrylase protein.

The expression cassette of the present invention was produced using methods well known in the art. Briefly this involves the steps of:

1. Isolation and cloning of the sheep metallothionein-Ia promoter sequence.
2. Isolation and modification of the bacterial coding sequence and fusion to the bacterial coding sequence.
3. Fusion of exon 5 of the sheep growth hormone gene to the metallothionein promoter/bacterial coding sequence complex.

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In order to provide further details on construction of the cassette the procedure followed in construction of MTCE10 was as follows:

Step 1.

- 5 A bacterial plasmid containing the sheep metallothionein-Ia gene was digested with the restriction enzymes Eco RI and BamHI and a DNA fragment encoding the promoter region of the gene separated by agarose gel electrophoresis and cloned in the plasmid vector pUC8.

10 Step 2.

- The coding sequence and associated 5' and 3' DNA encompassing the cyse gene of Escherichia coli was cloned in the plasmid vector pGEM3 as an Eco RI fragment excised from a lambda transducing phage containing portion of the
- 15 E.coli chromosome. Sub-fragments of this insert were then cloned into the bacteriophage M13 and the clones encompassing the bacterial initiation codon and the bacterial stop codon were used for site-directed mutagenesis to introduce a Bam HI site at the 5' end of
- 20 the coding sequence and a Sau 3A site at the 3' end of the gene. The mutagenesis was carried out on single-strand DNA by conventional procedures and the resulting modified DNA used to replace the corresponding DNA fragments in the insert of the original pGEM3 clone. A Bam HI - Sau 3A
- 25 fragment of DNA was then excised from this plasmid and inserted into a similarly digested sample of the plasmid containing the metallothionein-Ia sequence.

Step 3.

- The plasmid containing the metallothionein-Ia promoter-csyE coding sequence was digested with Pvu II
- 30 (adjacent to the introduced Sau 3A site) and to this was ligated a blunt-ended Pst I DNA fragment isolated from the sheep growth hormone gene and encompassing exon 5. Plasmids containing the correct orientation of the growth
- 35 hormone sequence were identified by restriction enzyme mapping.

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GENE DETAILS

Gene 1 (MTCE10)

This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the Escherichia coli cysE gene at a unique BamHI restriction enzyme site. This sequence was then joined to the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification in the vicinity of the initiation and stop codons of the bacterial cysE gene were made by site-directed mutagenesis using synthetic oligonucleotides. The metallothionein promoter replaces all regulatory sequences located 5' to the cysE gene coding sequence, and the growth hormone exon 5 sequence replaces all untranslated sequences located 3' to the cysE gene coding sequence. The gene is approximately 3580 base pairs in length, of which 2827 nucleotides have been sequenced. The sequence of gene 1 is shown in Figure 2.

Gene 2 (MTCK7)

This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the Escherichia coli cysK gene at a unique Sal I restriction enzyme site. This sequence was then joined to the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification of the cysK gene in the vicinity of the initiation codon was made by site-directed mutagenesis using a synthetic oligonucleotide. The metallothionein promoter replaces all regulatory sequences located 5' to the cysK coding sequence, and the sheep growth hormone exon 5 replaces all untranslated sequence located 3' to the cysK coding sequence. The size of the gene is approximately 3750 base pairs in length, of which 2957 base pairs have been sequenced. The sequence of gene 2 is shown in Figure 3.

Gene 3 (MTCEK1)

This gene consists of a fusion of genes 1 and 2 to

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create a single DNA sequence that encodes both the serin transacetylase and the O-acetylserine sulphydrylase enzymes. Each coding sequence is separately regulated by its own adjacent sheep metallothionein-Ia gene promoter sequence, and each coding sequence is separately followed by the 3' sequence of exon 5 of the sheep growth hormone gene. The gene is approximately 7550 base pairs in size, of which 5784 nucleotides have been sequenced. The sequence of gene 3 is shown in Figure 4.

10 Example 2. The expression of the glyoxylate cycle in transgenic animals

In order to provide the enzymes needed for the operation of the glyoxylate cycle in transgenic animals, the E. coli genes encoding the enzymes isocitrate lyase and malate synthase have been inserted into the expression cassette.

Three genes are described. Genes 1 and 2 each encode single bacterial proteins, gene 1 encoding the protein isocitrate lyase and gene 2 encoding the protein malate synthase. Gene 3 is a compound gene constructed from gene 1 and gene 2, and encodes both the isocitrate lyase and the malate synthase proteins.

GENE DETAILS

Gene 4 (MTAceA2)

25 This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the Escherichia coli aceA gene at a unique BamHI restriction enzyme site. This sequence was then joined to the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification in the vicinity of the initiation and stop codons of the bacterial aceA gene were made by site-directed mutagenesis using synthetic oligonucleotides. The metallothionein promoter replaces all regulatory sequences located 5' to the aceA gene coding sequence, and the growth hormone exon 5 sequence

30

35

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replaces all untranslated sequences located 3' to the aceA gene coding sequence. The gene is approximately 3580 base pairs in length, of which 2827 nucleotides have been sequenced. The sequence of gene 4 is shown in Figure 5.

5 Gene 5 (MTAceB2)

This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the Escherichia coli aceB gene at a unique Sal 1 restriction enzyme site. This sequence was then joined to
10 the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification of the aceB gene in the vicinity of the initiation codon was made by site-directed mutagenesis using a synthetic oligonucleotide. The metallothionein promoter replaces all regulatory sequences
15 located 5' to the aceB coding sequence, and the sheep growth hormone exon 5 sequence replaces all untranslated sequence located 3' to the aceB coding sequence. The size of the gene is approximately 3750 base pairs in length, of which 2957 base pairs have been sequenced. The sequence
20 of gene 5 is shown in figure 6.

Gene 6 (MTAceAB1)

This gene consists of a fusion of genes 1 and 2 to create a single DNA sequence that encodes both the isocitrate lyase and the malate synthase enzymes. Each
25 coding sequence is separately regulated by its own adjacent sheep metallothionein-Ia gene promoter sequence, and each coding sequence is separately followed by the 3' sequence of exon 5 of the sheep growth hormone gene. The gene is approximately 7550 base pairs in size, of which
30 5784 nucleotides have been sequenced. The sequence of gene 6 is shown in Figure 7.

REGULATION OF THE GENES

Regulation in Cultured Cells

Genes 1 to 6 have been transfected into mouse L-cells

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in cultur to produc stably transformed cell lines. The
xpression of ach gene was measured by:

1. Northern blot analysis of extracted RNA.
2. Enzyme assay of cell extracts.

5 An RNA transcript of the expected size was detected
in RNA extracted from each cell line, using a probe
specific for the appropriate coding sequence of each
gene. The intensity of the hybridisation increased when
cells were grown in a medium containing 10 uM zinc
10 sulphate, indicating that the genes were regulated by
heavy metals.

The results of enzyme assays of cell extracts from
each of the transformed cell lines are shown in Table 1
(genes 1 - 3) and Table 4 (genes 4,5). High levels of
15 activity of serine transacetylase, O-acetylserine
sulphydrylase, isocitrate lyase and malate synthase were
measured in the appropriate cell extracts, and the enzyme
levels were increased when cells were grown in
zinc-supplemented growth media.

20 Cell extracts prepared from cells containing the
fusion gene MTCEK1 contained both serine transacetylase
and O-acetylserine sulphydrylase enzyme activities,
indicating that both coding sequences within the fusion
gene were transcribed and translated. Furthermore, when
25 extracts from this cell line were incubated with the
substrates serine and H₂S, substantial quantities of
cysteine were produced, evidence that the entire
biochemical pathway is operational in these cells.
Similarly, cell extracts prepared from the cells
30 containing the fusion gene MTAcEAB1 contained both
isocitrate lyase and malate synthase enzyme activities,
indicating that both coding sequences within the fusion
gene were transcribed and translated.

Expression in Transgenic Mice

35 Genes 1 to 6 were each transferred to transgenic mice

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by the technique of single-cell embryo pronuclear microinjection. Mice containing the new genes were analyzed for expression by extracting mRNA and preparing cell-free supernatants from various tissues including liver, kidney and intestine. As shown in Tables 3 and 5, high levels of activity of the various enzymes were detected in appropriate transgenic mice. Furthermore, the expression of the genes in the intestinal tissues was highly zinc-dependent.

10 TABLE 2

Expression of MTCE10 and MTCK7 in transformed mouse L-cells

		<u>Serine Transacetylase</u>		<u>O-acetylserine</u>	
				<u>Sulphydrylase</u>	
cells		-Zn	+Zn	-Zn	+Zn
15	control	0	0	0	0
	MTCE10	1281	2706	-	-
	MTCK7	-	-	38	1367
	MTCEK1	120	360	1082	7790
20	Values are nmoles product formed/mg protein/30 min				

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TABLE 3

Activity of serine transacetylase (SAT) and O-acetylserine sulphydrylase (OAS) in tissue extracts prepared from transgenic mice. CK7-26 contains the gene pMTCK7, CE10-29 contains pMTCE10 and CEK1-28 and CEK1-8 contains pMTCEK1. Specific activity is measured as nmoles substrate utilised (SAT) or product formed (OAS/30 min/mg protein).

	<u>MOUSE LINE</u>	<u>ORGAN</u>	<u>SAT</u>	<u>OAS</u>
10	CK7-26	Intestine	-	206
		Kidney	-	352
		Liver	-	13
	CE10-29	Intestine	6,546	-
		Kidney	0	-
		Liver	0	-
15	CEK1-28	Intestine	1,161	2,797
		Kidney	0	24
		Liver	0	3
		Brain	16	86
20	CEK1-8	Intestine	4,522	12,778
		Kidney	105	128
		Liver	9	3
		Brain	0	245
			0	158
25		Skin	0	329
			6	295

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In order to assess the ability of transgenic mice containing the pMTCEK1 gene to produce cysteine, transgenic mice including this gene and control mice were given 25 mM ZnSO₄ in their drinking water for a minimum of four days. On the day of the experiment the ZnSO₄ was replaced with normal drinking water and 60 min. later 30 - 60 uCi of Na₂³⁵S was administered per os. The mice were sacrificed 60 min. later and intestinal tissue homogenised in a buffered aqueous solution containing 10mM dithiothreitol. Two volumes of performic acid were then added and the solution left at room temperature overnight. The suspension was then extracted with chloroform/methanol by conventional means and the aqueous layer concentrated by evaporation. Aliquots of the solution were then placed on Whatman 3mm filter paper and subjected to electrophoresis in a solution of pyridine:acetic acid:H₂O (10:100:900, pH3.6) at a voltage of 200 Volts for 2 hr. The paper was then cut into 0.5 cm strips and radioactivity counted in a scintillation counter under standard conditions. The results are shown in Figure 8. As can be seen from these results the transgenic mice were able to synthesise radiolabelled cysteine from the administered sodium sulphide in contrast to the control mice.

25 TABLE 4

Expression of MTAceA2 and MTAceB2 in transformed mouse L-cells

cell line	isocitrate lyase	malate synthase
control	0	0
30 MTAceA2	68	-
MTAceB2	-	34.3

Values are nmoles product/mg protein/20 min

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TABLE 5

Expression of MTaceAB1 in transgenic mice

<u>Mouse</u>	<u>Tissue</u>	<u>Isocitrate Lyase</u>	<u>Malate Synthase</u>
5	control intestine	not detectable	not detectable
	liver	not detectable	not detectable
	kidney	not detectable	not detectable
10	MTaceAB1 intestine	27.2	ND
	liver	not detectable	182
	kidney	not detectable	1.6

Values of isocitrate lyase are nmoles product/mg protein/20 min, and for malate synthase are picomoles product/mg protein/20 min ($\times 10^{-2}$)

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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CLAIMS:-

1. A genetic expression cassette for use in obtaining expression of a cDNA sequence in animal cells, the cassette comprising an inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene or a portion thereof, the cDNA sequence being positioned between the inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene.
2. A genetic expression cassette as claimed in claim 1 in which the inducible promoter is the immediate upstream nucleotide sequence of the sheep metallothionein-Ia gene.
3. A genetic expression cassette as claimed in claim 1 or claim 2 in which the cDNA codes for a bacterial enzyme, plant chitinase, insecticidal scorpion vermon toxin or the insecticidal protein of Bacillus thuringiensis.
4. A genetic expression cassette as claimed in claim 3 in which the cDNA sequence is selected from the group consisting of cysE, cysK, aceA and aceB genes of Escherichia coli.
5. A genetic expression cassette as claimed in claim 1 in which the expression cassette has a sequence substantially as shown in Figure 1.
6. A transgenic non-human animal including the genetic expression cassette as claimed in any one of claims 1 to 5.
7. A transgenic non-human animal as claimed in claim 6 in which the animal is ovine or bovine.

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FIG. 1 1/2

SEQUENCE OF THE EXPRESSION CASSETTE

1 metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctgggtatgtaattc
61
tcaggactattcaaagggaaatacccactgtcttacttcgttattgggatgccagctctgc
121
ccatcacttacaaggatgcttttcttagggggcattcctatgactagggaaacctccatcct
181
ggagccgggtggactggctaggcagtgattccctggcccattcatctattcagtcgtgg
241
agaatgtaaggaaggctgggacagagaaggctgagttcgctgctgggctgttacaggaga
301
aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
361
gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
481
gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
541
gttcccgtaaacaccgatgactgccacattgtggaaagctgggaagggggcgggcaggaa
601
tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggagtaggggg
661
acggaggcggttcgggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
721
cgcggtggtgctcacgcggccgacccgggtgcagcgggcagctcgggtgcaggcgggggag
781 metallothionein cap site *
accctctgcgcccggccgcctcctgtgggtataatagcgtcggctcctgggctccaac
841
acgcctcccaccggaccagtggatccaca INSERT GENE IN THIS POSITION
910 growth hormone exon 5
tgtcctgtgatctaattgtcctgtgatcccgtgcgccttctagttgcca
960
gccatctgctgttaccctccctgtgccttcttagaccctggaaggtgccactccagtgc
1020
ccaccgtcctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcat
1080
tctattctagggggtggggtcgggcaggatagcgagggggaggattgggaagacaatagc
1140
aggggtgctgtgggctctatgggtaccaggtgctgaataattgaccgggtcctcctgg
1200
ggcagaaagaagcaggcacatccccttctctgtgacacaccgggtcctcgcccctggtec
1260
ttagttccagccccactcataggacactcacagctcaggaggggtccgccttcaatccca
1320
cccgtctaaagtgttggagcgggtctctccctctcagccaccagccgaatctaggcctcca

2/25

FIG. 1 2/2

1380
gagtgggaagaattttaagcaagacaggctatgaagtacagagggagagaaaaatgcctcca
1440
acatgtgaggaagtgatgagagaaagcgtagaattagttttgtggcataaattttaagggt
1500
gactacacacttggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtg
1560
tccagctctttgtgacccacggactgtggctgccaggctcctctgtccatgggattctc
1620
cagggcaagaatactggaggggggttgccattccccaggggatcttcccagcccaaggatc
1680
aaacccgagtttctgcattgcaggcagattctttactctctgagccatcagggaagccct
1740
gtgggaaatgggaaccatgcaagaatggctttgggaccaataggaccagaatgtttggga
1800
tctgaactgggtcaagagatgtggaagagagattctaaatgcatgtgttcatgctaagt
1860
gcttcagtcgtgtcctactatttgcaaccccgatgaactgcagccaccaggctcctctgt
1920
catgggattctccattcaagaatactggagtgaagtttcttctccccaggggatctcca
1980
aaccagggattgaccaggatctcttgtatctcctggcacttgacaggcaaattctctcac
2040
cactagcgccactggacccagtctaag--unsequenced region

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FIG. 2 1/3

SEQUENCE OF THE MTCE10 GENE

1 metallothionein promoter
 gaattcaaagaggaaaagtgatgaaacaaggccttggcacagactccctgggtatgtaattc
 61
 tcaggactattcaaagggaaatacccactgtcttacttctgttattggatgccagctctgc
 121
 ccatcacttacaaggatgcttttctagggggcatcctatgactaggggaacctccatcct
 181
 ggagccgggtggactggctaggcagtggttccctggcccattcatctattcagtcgtgg
 241
 agaatgtaaggaaggctgggacgacagaaggctgagttcgctgctgggctgttacaggaga
 301
 aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattaggggaagcg
 361
 ggggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
 421
 ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
 481
 ggggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcaggggactgg
 541
 gttcccgtaaacaccgatgactgccacattgtggaaagctgggaagggggcgggcaggaa
 601
 tcctggagcgctacttgtcattcgggacaaagtcctcccgcttgggggagtaggggg
 661
 acggaggcggttctcgggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
 721
 cgcgtggtgctcaccgcccgaaccgggtgcagcgggcagctcgggtgcaggcgggggag
 781
 accctctgcgcccggcccgcctcctgtgggtataatagcgctcgggtcctggggtccaac
 841
 bacterial cysE gene
 MetSerCysGluGluLeuGluIleValTrpA
 acgcctcccaccggaccagtggatccacaATGTCGTGTGAAGAACTGGAAATTGCTCTGGA
 901
 snAsnIleLysAlaGluAlaArgThrLeuAlaAspCysGluProMetLeuAlaSerPheT
 ACAATATTAAAGCCGAAGCCAGAACGCTGGCGGACTGTGAGCCAATGCTGGCCAGTTTTT
 961
 yrHisAlaThrLeuLeuLysHisGluAsnLeuGlySerAlaLeuSerTyrMetLeuAlaA
 ACCACGCGACGCTACTCAAGCACGAAAACCTTGGCAGTGCACTGAGCTACATGCTGGCGA
 1021
 snLysLeuSerSerProIleMetProAlaIleAlaIleArgGluValValGluGluAlaT
 ACAAGCTGTCATCGCCAATTATGCCTGCTATTGCTATCCGTGAAGTGGTGAAGAAGCCT
 1081
 yrAlaAlaAspProGluMetIleAlaSerAlaAlaCysAspIleGlnAlaValArgThrA
 ACGCCGCTGACCCGGAATGATCGCCTCTGCGGCCTGTGATATTCAGGCGGTGCGTACCC
 1141
 rgAspProAlaValAspLysTyrSerThrProLeuLeuTyrLeuLysGlyPheHisAlaL
 GCGACCCGGCAGTCGATAAATACTCAACCCGTTGTTATACCTGAAGGGTTTTTCATGCCT
 1201
 euGlnAlaTyrArgIleGlyHisTrpLeuTrpAsnGlnGlyArgArgAlaLeuAlaIleP
 TGCAGGCCTATCGCATCGGTCACTGGTTGTGGAATCAGGGGCGTCGCGCACTGGCAATCT
 1261
 heLeuGlnAsnGlnValSerValThrPheGlnValAspIleHisProAlaAlaLysIleG

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FIG. 2 2/3

TTCTGCAAAACCAGGTTTCTGTGACGTTCCAGGTCGATATTCACCCGGCAGCAAAAATTG
1321
lyArgGlyIleMetLeuAspHisAlaThrGlyIleValValGlyGluThrAlaValIleG
GTCGCGGTATCATGCTTGACCACGCGACAGGCATCGTCGTTGGTGAAACGGCGGTGATTG
1381
luAsnAspValSerIleLeuGlnSerValThrLeuGlyGlyThrGlyLysSerGlyGlyA
AAAACGACGTATCGATTCTGCAATCTGTGACGCTTGGCGGTACGGGTAAATCTGGTGGTG
1441
spArgHisProLysIleArgGluGlyValMetIleGlyAlaGlyAlaLysIleLeuGlyA
ACCGTCACCCGAAAATTTCGTGAAGGTGTGATGATTGGCGCGGGCGCGAAAATCCTCGGCA
1501
snIleGluValGlyArgGlyAlaLysIleGlyAlaGlySerValValLeuGlnProValP
ATATTGAAGTTGGGCGCGGCGCGAAGATTGGCGCAGGTTCCGTGCTGCTGCAACCGGTGC
1561
roProHisThrThrAlaAlaGlyValProAlaArgIleValGlyLysProAspSerAspL
CGCCGCATACCACCGCCGCTGGCGTTCGGGCTCGTATTGTGCGGTAAACCAGACAGCGATA
1621
ysProSerMetAspMetAspGlnHisPheAsnGlyIleAsnHisThrPheGluTyrGlyA
AGCCATCAATGGATATGGACCAGCATTTCAACGGTATTAACCATACATTTGAGTATGGGG
1681
spGlyIle*** growth hormone exon 5
ATGGGATCTAAAtgtcctgtgatctaattgtcctgtgatcccgctgcgcttctagtgtcca
1741
gccatctgctgttaccctccctgtgccttcctagaccctggaaggtgccactccagtgc
1801
ccaccgtcctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcat
1861
tctattctagggggtggggtcgggcaggatagcgagggggaggattgggaagacaatagc
1921
aggggtgctgtgggctctatgggtaccaggtgctgaataattgaccgggttcctcctgg
1981
ggcagaaagaagcaggcacatcccttctctgtgacacaccgggtcctcgcccttgggtcc
2041
ttagttccagccccactcataggacactcacagctcaggagggtccgccttcaatccca
2101
cccgctaaagtgttgaggcgggtctctccctctcagccaccagccgaatctaggcctcca
2161
gagtgggaagaatttaagcaagacaggctatgaagtacagagggagagaaaatgcctcca
2221
acatgtgaggaagtgtgagagaaagcgtagaattagttttgtggcataaattttaaggt
2281
gactacacacttggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtg
2341
tccagctctttgtgacccacaggactgtgggtgccagggtcctctgtccatgggattctc
2401
cagggaagaataactggaggggggttgccattccccaggggatcttcccagcccaaggatc
2461
aaaccgagtttctgcattgcaggcagattctttactctctgagccatcagggaagccct
2521
gtgggaaatgggaaccatgcaagaatggctttgggaccaataggaccagaatgtttggga
2581
tctgaactgggtcaagagatgtggaagagagattctaaatgcatgtgttcattgctaagtg

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FIG. 2 3/3

2641
gcttcagtcgtgtcctactatTTTgcaaccccgatgaactgcagccaccaggctcctctgt
2701
catgggattctccattcaagaatactggagtgagtttccttcctccccaggggatctcca
2761
aaccagggattgaccaggatctcttgtatctcctggcacttgacaggcaaattctctcac
2821
cactagcgccactggacccagTctaag--unsequenced region

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FIG. 3 1/3

SEQUENCE OF THE MTCK7 GENE

1 metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggatatgtaattc
61
tcaggactattcaaagggaaatacccactgtcttacttcgttattggatgccagctctgc
121
ccatcacttacaaggatgcttttcctagggggcatcctatgactagggaaacctccatcct
181
ggagccgggtggactggctaggcagtggttccctggcccattcatctattcagtcgtgg
241
agaatgtaaggaaggctggggcgacagaaggctgagttcgctgctgggctgttacaggaga
301
aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattaggggaagcg
361
gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
481
gggtgaaagcaaagacaagagttgcgggggcaggaagactgcgaggactcagggactgg
541
gttcccgtaaacaccgatgactgccacattgtggaaagctgggaagggggcgggcaggaa
601
tcctggagcgctacttgtcattcgggacaaaagtcctcccgcttgggggagtaggggg
661
acggaggcggttcgggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
721
cgcggtggtgctcaccgccccgaccgggtgcagcgggcagctcgggtgcaggcgggggag
781
accctctgcgcccggccccgcctcctgtgggtataatagcgctcgggtcctgggctccaac
841
bacterial cysK gene
MetSerLysIlePheGluAsnSer
acgcctcccaccggaccagtggatccgtcgaccATGAGTAAGATTTTGAAGATAACTCG
901
LeuThrIleGlyHisThrProLeuValArgLeuAsnArgIleGlyAsnGlyArgIleLeu
CTGACTATCGGTCACACGCCGCTGGTTCGCCTGAATCGCATCGGTAACGGACGCATTCTG
961
AlaLysValGluSerArgAsnProSerPheSerValLysCysArgIleGlyAlaAsnMet
GCGAAGGTGGAATCTCGTAACCCAGCTTCAGCGTTAAGTGCCGTATCGGTGCCAACATG
1021
IleTrpAspAlaGluLysArgGlyValLeuLysProGlyValGluLeuValGluProThr
ATTTGGGATGCCGAAAAGCGCGGCGTGCTGAAACCAGGCGTTGAAGTGGTTGAACCGACC
1081
SerGlyAsnThrGlyIleAlaLeuAlaTyrValAlaAlaAlaArgGlyTyrLysLeuThr
AGCGGTAATACGGGATTGCACTGGCCTATGTAGCTGCCGCTCGCGGTTACAAACTCACC
1141
LeuThrMetProGluThrMetSerIleGluArgArgLysLeuLeuLysAlaLeuGlyAla
CTGACCATGCCAGAAACCATGAGTATTGAACGCCGCAAGCTGCTGAAAGCGTTAGGTGCA
1201
AsnLeuValLeuThrGluGlyAlaLysGlyMetLysGlyAlaIleGlnLysAlaGluGlu
AACCTGGTGCTGACGGAAGGTGCTAAAGGCATGAAAGGCGCAATCCAAAAGCAGAAGAA

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FIG. 3 2/3

1261
IleValAlaSerAsnProGluLysTyrLeuLeuLeuGlnGlnPheSerAsnProAlaAsn
ATTGTCGCCAGCAATCCAGAGAAATACCTGCTGCTGCAACAATTCAGCAATCCGGCAAAC
1321
ProGluIleHisGluLysThrThrGlyProGluIleTrpGluAspThrAspGlyGlnVal
CCTGAAATTCACGAAAAGACCACCGGTCCGGAGATATGGGAAGATACCGACGGTCAGGTT
1381
AspValPheIleAlaGlyValGlyThrGlyGlyThrTrpThrGlyValThrProTyrIle
GATGTATTTATTGCTGGCGTTGGGACTGGCGGTACGTGGACTGGCGTCACGCCCTACATT
1441
LysGlyThrLysGlyLysThrAspLeuIleSerValAlaValGluProThrAspSerPro
AAAGGCACCAAAGGCAAGACCGATCTTATCTCTGTGCGCGTTGAGCCAACCGATTCTCCA
1501
ValIleAlaGlnAlaLeuAlaGlyGluGluIleLysProGlyProHisLysIleGlnGly
GTTATCGCCCAGGCGCTGGCAGGTGAAGAGATTAAACCTGGCCCCGATAAAATTCAGGGT
1561
IleGlyAlaGlyPheIleProAlaAsnLeuAspLeuLysLeuValAspLysValIleGly
ATTGGCGCTGGTTTTATCCCGGCTAACCTCGATCTCAAGCTGGTCGATAAAGTCATTGGC
1621
IleThrAsnGluGluAlaIleSerThrAlaArgArgLeuMetGluGluGluGlyIleLeu
ATCACCAATGAAGAAGCGATTCTACCGCGCGTCTGATGGAAGAAGAAGGTATTCTT
1681
AlaGlyIleSerSerGlyAlaAlaValAlaAlaAlaLeuLysLeuGlnGluAspGluSer
GCAGGTATCTCTTCTGGAGCAGCTGTTGCCGCGGCGTTGAAACTACAAGAAGATGAAAGC
1741
PheThrAsnLysAsnIleValValIleLeuProSerSerGlyGluArgTyrLeuSerThr
TTTACCAACAAGAATATTGTGGTTATTCTACCATCATCGGGTGAGCGTTATTTAAGCACC
1801
AlaLeuPheAlaAspLeuPheThrGluLysGluLeuGlnGln*** growth hormone
GCATTGTTTGCCGATCTCTTCACTGAGAAAGAATTGCAACAGTAAtggccagctgcgcct
1861 exon 5
tctagtgtgccagccatctgctgttaccctccctgtgccttcttagaccctggaaggtgc
1921
cactccagtgcccaccgtcctttcttaataaagcggaggaaattgcatcacattgtctga
1981
gtaggtgtcattctattctaggggggtgggggtcgggcaggatagcgagggggaggattggg
2041
aagacaatagcaggggtgctgtgggctctatgggtacccaggtgctgaataattgacccg
2101
gttcctcctggggcgagaaagaagcaggcacatcccttctctgtgacacacccggtcctc
2161
gcccctggtccttagttccagccccactcataggacactcacagctcaggaggggtccgc
2221
cttcaatcccaccogctaaagtgcttgagcgggtctctccctctcagccaccagccgaat
2281
ctaggcctccagagtgggaagaatttaagcaagacaggctatgaagtacagagggagaga
2341
aaatgcctccaacatgtgaggaagtgatgagagaaagcgtagaattagttttgtggcata
2401
aattttaaggtgactacacacttgcccaactacccttgggaaatgtgtgtgtgttagtc
2461
actcagttgtgtccagctctttgtgacccacggactgtggctgccagggtcctctgtcc

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FIG. 3 3/3

2521
atgggattctccagggcaagaatactggagggggttgccattccccaggggatcttccca
2581
gccaaggatcaaaccgagtttctgcattgcaggcagattctttactctctgagccatc
2641
aggggaagccctgtgggaaatgggaaccatgcaagaatggctttgggaccaataggaccag
2701
aatgtttgggatctgaactgggtcaagagatgtggaagagagattctaaatgcatgtgtt
2761
catgctaagtggcttcagtcgtgtcctactatttgcaaccccgatgaactgcagccacca
2821
ggctcctctgtcatgggattctccattcaagaatactggagtgagtttccttcctcccca
2881
ggggatctccaaaccagggattgaccaggatctcttgatatctcctggcacttgacaggc
2941
aaatctctcaccactagcgccactggaccagtctaag---unsequenced region

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FIG. 4 1/5

SEQUENCE OF THE MTCEK1 GENE

1 metallothionein promoter
 atcatcgatcaggcagaattcaaagaggaaaagtgatgaaacaaggcttggcacagactc
 61
 cctggtatgtaattctcaggactattcaaagggaaatacccactgtcttacttcgttatt
 121
 ggatgccagctctgcccataccttacaaggatgcttttcttagggggcctcctatgacta
 181
 gggaacctccatcctggagccgggtggactggctaggcagtgattccctggccattca
 241
 tctattcagtcgtggagaatgtaaggaaggctgggacagagaaggctgagttcgtcgtc
 301
 ggctgttacaggagaaactagagactctgttcaaagtccagggtgggggctgtgggagga
 361
 aatattaggaagcgggggttcgggggataggtggtgaagctcacatccatcacgggtctc
 421
 tgcacacgacacaggggctccagccaagcctgggatgtgagcacgaggctcggattgcgc
 481
 atgagctctgggaaaggggtgaaagcaaagacaagagttgcgggggcagggaagactgcga
 541
 ggactcagggaactgggttcccgtaaacaccgatgactgcccacattgtggaaagctggga
 601
 aggggcgggcaggaatcctggagcgtacttgtcattcgggacaaagtccctccgcgttg
 661
 ggggagagtagggggacggaggcggttccggtgcgcacggagcccagccgcgttccgggaa
 721
 tcttgcgctcggccgcgcgtggtgctcaccgcccagcccgggtgcagcgggcagctcggg
 781
 tgcaggcgggggcagaccctctgcgcccggccgcctcctgtgggtataatagcgctcgg
 841
 bacterial cysE
 gene

* metallothionein cap site - MetSerCysGluGluL
 ctccctgggctccaacacgcctcccaccggaccagtggatccacaATGTCGTGTGAAGAAC
 901
 euGluIleValTrpAsnAsnIleLysAlaGluAlaArgThrLeuAlaAspCysGluProM
 TGGAAATTGTCTGGAACAATATTAAAGCCGAAGCCAGAACGCTGGCGGACTGTGAGCCAA
 961
 etLeuAlaSerPheTyrHisAlaThrLeuLeuLysHisGluAsnLeuGlySerAlaLeuS
 TGCTGGCCAGTTTTTACCACGCGACGCTACTCAAGCACGAAAACCTTGGCAGTGCCTGA
 1021
 erTyrMetLeuAlaAsnLysLeuSerSerProIleMetProAlaIleAlaIleArgGluV
 GCTACATGCTGGCGAACAAGCTGTCATCGCCAATTATGCCTGCTATTGCTATCCGTGAAG
 1081
 alValGluGluAlaTyrAlaAlaAspProGluMetIleAlaSerAlaAlaCysAspIleG
 TGGTGGGAAGAAGCCTACGCCGCTGACCCGGAAATGATCGCCTCTGCGGCCTGTGATATTC
 1141
 lnAlaValArgThrArgAspProAlaValAspLysTyrSerThrProLeuLeuTyrLeuL
 AGGCGGTGCGTACCCGCGACCCGGCAGTCGATAAATACTCAACCCCGTTGTTATACCTGA
 1201
 ysGlyPheHisAlaLeuGlnAlaTyrArgIleGlyHisTrpLeuTrpAsnGlnGlyArgA
 AGGGTTTTTCATGCCTTGCAGGCCTATCGCATCGGTCACTGGTTGTGGAATCAGGGGCGTC

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FIG. 4 2/5

1261
rgAlaLeuAlaIlePheLeuGlnAsnGlnValSerValThrPheGlnValAspIleHisP
GCGCACTGGCAATCTTTCTGCAAACACAGGTTTCTGTGACGTTCCAGGTCGATATTCACC
1321
roAlaAlaLysIleGlyArgGlyIleMetLeuAspHisAlaThrGlyIleValValGlyG
CGGCAGCAAAAATTGGTCGCGGTATCATGCTTGACCACGCGACAGGCATCGTCGTTGGTG
1381
luThrAlaValIleGluAsnAspValSerIleLeuGlnSerValThrLeuGlyGlyThrG
AAACGGCGGTGATTGAAAACGACGTATCGATTCTGCAATCTGTGACGCTTGGCGGTACGG
1441
lyLysSerGlyGlyAspArgHisProLysIleArgGluGlyValMetIleGlyAlaGlyA
GTAAATCTGGTGGTGACCGTCACCCGAAAATTCTGTAAGGTGTGATGATTGGCGCGGGCG
1501
laLysIleLeuGlyAsnIleGluValGlyArgGlyAlaLysIleGlyAlaGlySerValV
CGAAAATCCTCGGCAATATTGAAGTTGGGCGCGGCGGAAGATTGGCGCAGGTTCCGTGG
1561
alLeuGlnProValProProHisThrThrAlaAlaGlyValProAlaArgIleValGlyL
TGCTGCAACCGGTGCCGCCGCATACCACCGCCGCTGGCGTTCCGGCTCGTATTGTCGGTA
1621
ysProAspSerAspLysProSerMetAspMetAspGlnHisPheAsnGlyIleAsnHist
AACCAGACAGCGATAAGCCATCAATGGATATGGACCAGCATTCAACGGTATTAACCATA
1681
hrPheGluTyrGlyAspGlyIle*** growth hormone exon 5
CATTTGAGTATGGGGATGGGATCTAATgtcctgtgatctaattgtcctgtgatcccgctgc
1741
gccttctagttgccagccatctgctgttaccctccctgtgccttcttagaccctggaag
1801
gtgccactccagtgcccaccgtcctttcttaataaagcggaggaaattgcatcacattgt
1861
ctgagtaggtgtcattctattctagggggtgggggtcgggcaggatagcgagggggaggat
1921
tggaagacaatagcaggggtgctgtgggctctatgggtaccaggtgctgaataattga
1981
cccggttctcctcctggggcagaaagaagcaggcacatccccttctctgtgacacaccgggt
2041
cctcgcccctggtccttagttccagccccactcataggacactcacagctcaggagggt
2101
ccgccttcaatcccacccgctaaagtgttgaggcgggtctctcctctcagccaccagcc
2161
gaatctaggcctccagagtggaagaatttaagcaagacaggctatgaagtacagagggga
2221
gagaaaatgcctccaacatgtgaggaagtgtgatgagagaaagcgtagaattagttttgtgg
2281
cataaattttaaggtgactacacacttggcccaactacccttgggaaatgtgtgtgtgtt
2341
agtcactcagttgtgtccagctctttgtgacccacggactgtgggtgccagggtcctct
2401
gtccatgggattctccagggaagaatactggaggggggttgcattcccaggggatcctt
2461
cccagcccaaggatcaaaccgagtttctgcattgcaggcagattctttactctctgagc
2521
catcaggaagccctgtgggaaatgggaaccatgcaagaatggctttgggaccaatagga

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FIG. 4 3/5

2581
ccagaatgtttgggatctgaactgggtcaagagatgtggaagagagattctaaatgcatg
2641
tggtcatgctaagtggcttcagtcgtgtcctactatattgcaaccccgatgaactgcaggc
2701 metallothionein promoter
atgcaagcttcagatcatcgatgaattcaaagagggaaaagtgatgaaacaaggcttgga
2761
cagactccctgggtatgtaattctcaggactattcaaagggaaataccactgtcttactt
2821
cgttattggatgccagctctgccatcacttacaaggatgcttttcttagggggcatcct
2881
atgactagggaaacctccatcctggagccgggtggactggctagggcagtggattccctggc
2941
ccattcatctattcagtcgtggagaatgtaaggaaggctgggcgacagaaggctgagttc
3001
gctgctgggctgttacaggagaaactagagactctgttcaaagtccagggtgggggctgt
3061
gggaggaaatattaggggaagcggggttcgggggataggtggtgaagctcacatccatcac
3121
gggtctctgcacacgacacaggggctccagccaagcctgggatgtgagcacgaggctcgg
3181
attgcgcatgagctctgggaaagggtgaaagcaaagacaagagttgcgggggcagggaag
3241
actgcgaggactcagggactgggttcccgtaaacaccgatgactgccacattgtggaaa
3301
gctgggaagggcgggcaggaatcctggagcgctacttgtcattcgggacaaagtccctc
3361
cgcgttgggggagtagggggacggaggcggttcggtgcgcacggagcccagccgcgtt
3421
ccgggaatcttgcgctcggccgcgcgtggtgctcaccgcccgaccgggtgcagcgggca
3481
gctcgggtgcaggcgggggcagaccctctgcgcccggccgcctcctgtgggtataatag
3541 bacterial cysK gene
* metallothionein cap site MetSe
cgctcggctcctgggctccaacacgcctcccaccggaccagtggatccgtcgaccATGAG
3601
rLysIlePheGluAspAsnSerLeuThrIleGlyHisThrProLeuValArgLeuAsnAr
TAAGATTTTTGAAGATAACTCGCTGACTATCGGTCACACGCCGCTGGTTCGCCTGAATCG
3661
gIleGlyAsnGlyArgIleLeuAlaLysValGluSerArgAsnProSerPheSerValLy
CATCGGTAACGGACGCATTCTGGCGAAGGTGGAATCTCGTAACCCAGCTTCAGCGTTAA
3721
sCysArgIleGlyAlaAsnMetIleTrpAspAlaGluLysArgGlyValLeuLysProGl
GTGCCGTATCGGTGCCAACATGATTGGGATGCCGAAAAGCGCGCGTGTGAAACCAGG
3781
yValGluLeuValGluProThrSerGlyAsnThrGlyIleAlaLeuAlaTyrValAlaAl
CGTTGAACTGGTTGAACCGACCAGCGGTAATACCGGGATTGCACTGGCCTATGTAGCTGC
3841
aAlaArgGlyTyrLysLeuThrLeuThrMetProGluThrMetSerIleGluArgArgLy
CGCTCGCGGTTACAACTCACCTGACCATGCCAGAAACCATGAGTATTGAACGCCGCAA

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FIG. 4 4/5

3901
sLeuLeuLysAlaLeuGlyAlaAsnLeuValLeuThrGluGlyAlaLysGlyMetLysGl
GCTGCTGAAAGCGTTAGGTGCAAACCTGGTGCTGACGGAAGGTGCTAAAGGCATGAAAGG
3961
yAlaIleGlnLysAlaGluGluIleValAlaSerAsnProGluLysTyrLeuLeuLeuGl
CGCAATCCAAAAAGCAGAAGAAATTGTGCCAGCAATCCAGAGAAATACCTGCTGCTGCA
4021
nGlnPheSerAsnProAlaAsnProGluIleHisGluLysThrThrGlyProGluIleTr
ACAATTCAGCAATCCGGCAAACCCTGAAATTCACGAAAAGACCACCGGTCCGGAGATATG
4081
pGluAspThrAspGlyGlnValAspValPheIleAlaGlyValGlyThrGlyGlyThrTr
GGAAGATACCGACGGTCAGGTGATGTATTTATTGCTGGCGTTGGGACTGGCGGTACGTG
4141
pThrGlyValThrProTyrIleLysGlyThrLysGlyLysThrAspLeuIleSerValAl
GACTGGCGTCACGCCCTACATTAAAGGCACCAAAGGCAAGACCGATCTTATCTCTGTGCGC
4201
aValGluProThrAspSerProValIleAlaGlnAlaLeuAlaGlyGluGluIleLysPr
CGTTGAGCCAACCGATTCTCCAGTTATCGCCCAGGCGCTGGCAGGTGAAGAGATTAAACC
4261
oGlyProHisLysIleGlnGlyIleGlyAlaGlyPheIleProAlaAsnLeuAspLeuLy
TGGCCCGCATAAAATTCAGGGTATTGGCGCTGGTTTTATCCCGGCTAACCTCGATCTCAA
4321
sLeuValAspLysValIleGlyIleThrAsnGluGluAlaIleSerThrAlaArgArgLe
GCTGGTCGATAAAGTCATTGGCATCACCAATGAAGAAGCGATTCTACCGCGCGTCGTCT
4381
uMetGluGluGluGlyIleLeuAlaGlyIleSerSerGlyAlaAlaValAlaAlaAlaLe
GATGGAAGAAGAAGGTATTCTTGCAGGTATCTCTTCTGGAGCAGCTGTTGCCGCGGCGTT
4441
uLysLeuGlnGluAspGluSerPheThrAsnLysAsnIleValValIleLeuProSerSe
GAAACTACAAGAAGATGAAAGCTTTACCAACAAGAAATATTGTGGTTATTCTACCATCATC
4501
rGlyGluArgTyrLeuSerThrAlaLeuPheAlaAspLeuPheThrGluLysGluLeuGl
GGGTGAGCGTTATTTAAGCACCGCATTGTTTGCCGATCTCTTCACTGAGAAAGAATTGCA
4561
nGln*** growth hormone exon 5
ACAGTAAtggccagctgcgcccttctagttgccagccatctgctgttaccctccctgtgc
4621
cttcctagaccctggaaggtgccactccagtgccaccgctcctttcttaataaagcggag
4681
gaaattgcatcacattgtctgagtaggtgtcattctattctaggggggtggggtcgggag
4741
gatagcgaggggggaggattgggaagacaatagcaggggtgctgtgggctctatgggtacc
4801
caggtgctgaataattgacccggttcctcctggggcagaaagaagcaggcacatcccctt
4861
ctctgtgacacacccggtcctcgcccttggtccttagttccagccccactcataggacac
4921
tcacagctcaggagggtccgccttcaatcccaccgctaagtgcttgagcggtctct
4981
ccctctcagccaccagccgaatctaggcctccagagtgggaagaatttaagcaagacagg

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FIG. 4 5/5

5041
ctatgaagtacagagggagagaaaaatgcctccaacatgtgaggaagtgatgagagaaagc
5101
gtagaattagttttgtggcataaattttaaggtgactacacacttgcccaactaccctt
5161
gggaaatgtgtgtgtgttagtcactcagttgtgtccagctctttgtgacccacggactg
5221
tggctgccaggctcctctgtccatgggattctccagggcaagaatactggagggggtgc
5281
cattccccaggggatcttcccagcccaaggatcaaaccgagtttctgcattgcaggcag
5341
attctttactctctgagccatcaggggaagccctgtgggaaatgggaaccatgcaagaatg
5401
gctttgggaccaataggaccagaatgtttgggatctgaactgggtcaagagatgtggaag
5461
agagattctaaatgcattgtgttcattgctaagtggcttcagtcgtgtcctactatttgcaa
5521
ccccgatgaactgcaggcatgcaagcttcagctgc

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FIG. 5 1/3

SEQUENCE OF THE MTaceA2 GENE

1 metallothionein promoter
 gaattcaaagagaaaagtgatgaaacaaggcttggcacagactccctgggtatgtaattc
 61
 tcaggactattcaaagggaaataccactgtcttacttcgttattggatgccagctctgc
 121
 ccatcacttacaaggatgcttttctagggggcatcctatgactaggaacctccatcct
 181
 ggagccgggtggactggctagggcagtggttccctggccattcatctattcagtcgtgg
 241
 agaatgtaaggaaggctgggacgacagaaggctgagttcgctgctgggctgttacaggaga
 301
 aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
 361
 ggggtcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
 421
 ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
 481
 ggggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
 541
 gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaaggggaggcaggaa
 601
 tcctggagcgctacttgtcattcgggacaaagtcctccgcgttgggggagtagggggg
 661
 acggaggcggttccggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
 721
 cgcggtggtgctcacgccccgacccgggtgcagcgggcagctcgggtgcaggcgggggag
 781
 accctctgcgcccggcccgccctcctgtgggtataatagcgctcggctcctgggctccaac
 841
 bacterial ace A sequence

MetLysThrArgThrGlnG

acgcctcccaccggaccagtggatcctctagagtcaccATGAAAACCCGTACACAAC
 901
 lnIleGluGluLeuGlnLysGluTrpThrGlnProArgTrpGluGlyIleThrArgProT
 AAATTGAAGAATTACAGAAAGAGTGGACTCAACCGCGTTGGGAAGGCATTACTCGCCCAT
 961
 yrSerAlaGluAspValValLysLeuArgGlySerValAsnProGluCysThrLeuAlaG
 ACAGTGGGAAGATGTGGTGAATACGCGGTTAGTCAATCCTGAATGCACGCTGGCGC
 1021
 lnLeuGlyAlaAlaLysMetTrpArgLeuLeuHisGlyGluSerLysLysGlyTyrIleA
 AACTGGGCGCAGCGAAAATGTGGCGTCTGCTGCACGGTGAGTCGAAAAAAGGCTACATCA
 1081
 snSerLeuGlyAlaLeuThrGlyGlyGlnAlaLeuGlnGlnAlaLysAlaGlyIleGluA
 ACAGCCTCGGCGCACTGACTGGCGGTCAGGCGCTGCAACAGGCGAAAGCGGTATTGAAG
 1141
 laValTyrLeuSerGlyTrpGlnValAlaAlaAspAlaAsnLeuAlaAlaSerMetTyrP
 CAGTCTATCTGTCTGGGATGGCAGGTAGCGGCGGACGCTAACCTGGCGGCCAGCATGTATC
 1201
 roAspGlnSerLeuTyrProAlaAsnSerValProAlaValValGluArgIleAsnAsnT
 CGGATCAGTCTCTATCCGGCAAACCTCGGTGCCAGCTGTGGTGGAGCGGATCAACAACA

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FIG. 5 2/3

1261
hrPheArgArgAlaAspGlnIleGlnTrpSerAlaGlyIleGluProGlyAspProArgT
CCTTCCGTCGTGCCGATCAGATCCAATGGTCCGCGGGCATTGAGCCGGGCGATCCGCGCT
1321
yrValAspTyrPheLeuProIleValAlaAspAlaGluAlaGlyPheGlyGlyValLeuA
ATGTCGATTACTTCCTGCCGATCGTTGCCGATGCGGAAGCCGGTTTTGGCGGTGTCCTGA
1381
snAlaPheGluLeuMetLysAlaMetIleGluAlaGlyAlaAlaAlaValHisPheGluA
ATGCCTTTGAACTGATGAAAGCGATGATTGAAGCCGGTGCAGCGGCAGTTCACCTCGAAG
1441
spGlnLeuAlaSerValLysLysCysGlyHisMetGlyGlyLysValLeuValProThrG
ATCAGCTGGCGTCAGTGAAGAAATGCGGTCACATGGGCGGCAAAGTTTTAGTGCCAACTC
1501
lnGluAlaIleGlnLysLeuValAlaAlaArgLeuAlaAlaAspValThrGlyValProT
AGGAAGCTATTTCAGAACTGGTCGCGGCGCGTCTGGCAGCTGACGTGACGGGCGTTCCAA
1561
hrLeuLeuValAlaArgThrAspAlaAspAlaAlaAspLeuIleThrSerAspCysAspP
CCCTGCTGGTTGCCCGTACCGATGCTGATGCGGCGGATCTGATCACCTCCGATTGCGACC
1621
roTyrAspSerGluPheIleThrGlyGluArgThrSerGluGlyPhePheArgThrHisA
CGTATGACAGCGAATTTATTACCGGCGAGCGTACCAGTGAAGGCTTCTTCCGTACTCATG
1681
laGlyIleGluGlnAlaIleSerArgGlyLeuAlaTyrAlaProTyrAlaAspLeuValT
CGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCT
1741
rpCysGluThrSerThrProAspLeuGluLeuAlaArgArgPheAlaGlnAlaIleHisA
GGTGTGAAACCTCCACGCCGGATCTGGAAGTGGCGCGTCGCTTGCACAAGCTATCCACG
1801
laLysTyrProGlyLysLeuLeuAlaTyrAsnCysSerProSerPheAsnTrpGlnLysA
CGAAATATCCGGGCAAACCTGCTGGCTTATACTGCTCGCCGTCGTTCAACTGGCAGAAAA
1861
snLeuAspAspLysThrIleAlaSerPheGlnGlnGlnLeuSerAspMetGlyTyrLysP
ACCTCGACGACAAAACCTATTGCCAGCTTCCAGCAGCAGCTGTCCGATATGGGCTACAAGT
1921
heGlnPheIleThrLeuAlaGlyIleHisSerMetTrpPheAsnMetPheAspLeuAlaA
TCCAGTTCATCACCTGGCAGGTATCCACAGCATGTGGTTCAACATGTTTGACCTGGCAA
1981
snAlaTyrAlaGlnGlyGluGlyMetLysHisTyrValGluLysValGlnGlnProGluP
ACGCTATGCCCAGGGCGAGGGTATGAAGCACTACGTTGAGAAAGTGCAGCAGCCGGAAT
2041
heAlaAlaAlaLysAspGlyTyrThrPheValSerHisGlnGlnGluValGlyThrGlyT
TTGCCCGCGCAAAGATGGCTATACCTTCGTATCTCACCAGCAGGAAGTGGGTACAGGTT
2101
yrPheAspLysValThrThrIleIleGlnGlyGlyAspValPheSerHisArgAlaAspA
ACTTCGATAAAGTGACGACTATTATTTCAGGGCGGCGACGTCTTCAGTCACCGCGCTGACC
2161
growth hormone exon 5
rgLeuHis***
GGCTCCACTGAagaatcgcagttctaatttgacctgcgccttctagttgccagccatctg
2221
ctggttaccctccctgtgccttcctagaccctggaaggtgccactccagtgccaccgctc
2281
ctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcattctattct

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FIG. 5 3/3

2341
agggggtgggggtcgggcaggatagcgagggggaggattgggaagacaatagcaggggtgc
2401
tgtgggctctatgggtacccaggtgctgaataattgacccggttcctcctggggcagaaa
2461
gaagcaggcacatccccttctctgtgacacacccggtcctcgcccctggtccttagttcc
2521
agccccactcataggacactcacagctcaggagggctccgccttcaatcccacccgctaa
2581
agtgcttggagcgggtctctccctctcagccaccagccgaatctaggcctccagagtggga
2641
agaatttaagcaagacaggctatgaagtacagagggagagaaaaatgcctccaacatgtga
2701
ggaagtgatgagagaaaagcgtagaattagttttgtggcataaattttaagggtgactacac
2761
acttggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtgtccagctc
2821
tttgtgacccacggactgtgggtgccagggtcctctgtccatgggattctccagggcaa
2881
gaatactggaggggggttgccattccccaggggatcttcccagcccaaggatcaaaccoga
2941
gtttctgcattgcaggcagattctttactctctgagccatcaggggaagccctgtgggaaa
3001
tgggaaaccatgcaagaatggctttgggaccaataggaccagaatgtttgggatctgaact
3061
gggtcaagagatgtggaagagagattctaaatgcatgtgttcatgctaagtggcttcagt
3121
cgtgtcctactatttgcaaccccgatgaactgcag

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FIG. 6 1/3

SEQUENCE OF THE MTAcB2 GENE

1 metallothionein promoter
 gaattcaaagaggaagaaagtgatgaaacaaggcttggcacagactccctggtatgtaattc
 61
 tcaggactattcaaagggaaataccactgtcttacttcgttattggatgccagctctgc
 121
 ccatacttacaaggatgcttttcctagggggcatcctatgactaggggaacctccatcct
 181
 ggagccgggtggactggctaggcagtggttccctggccattcatctattcagtcgtgg
 241
 agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
 301
 aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattaggggaagcg
 361
 gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
 421
 ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
 481
 ggggtgaaagcaaagacaagagttgcgggggcagggaagactgagaggactcaggggactgg
 541
 gttcccgtaaacaccgatgactgccacattgtggaaagctgggaagggggcgggcaggaa
 601
 tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggagtaggggg
 661
 acggaggcggtttcgggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
 721
 cgcgtggtgctcaccgccccgacccgggtgcagcgggcagctcgggtgcaggcgggggag
 781
 accctctgcgcccggcccgccctcctgtgggtataatagcgctcggctcctgggctccaac
 841
 bacterial aceB sequence

MetThrGluGlnAlaThrT
 acgcctcccaccggaccagtggtatcctctagagtcacaccATGACTGAACAGGCAACAA
 901
 hrThrAspGluLeuAlaPheThrArgProTyrGlyGluGlnGluLysGlnIleLeuThrA
 CAACCGATGAACCTGGCTTTCACAAGGCCGTATGGCGAGCAGGAGAAGCAAATTCTTACTG
 961
 laGluAlaValGluPheLeuThrGluLeuValThrHisPheThrProGlnArgAsnLysL
 CCGAAGCGGTAGAATTTCTGACTGAGCTGGTGACGCATTTTACGCCACAACGCAATAAAC
 1021
 euLeuAlaAlaArgIleGlnGlnGlnGlnAspIleAspAsnGlyThrLeuProAspPheI
 TTCTGGCAGCGCGCATTTCAGCAGCAGCAAGATATTGATAACGGAACGTTGCCTGATTTTA
 1081
 leSerGluThrAlaSerIleArgAspAlaAspTrpLysIleArgGlyIleProAlaAspL
 TTTCGGAAACAGCTTCCATTTCGCGATGCTGATTGGAAAATTCGCGGGATTCCTGCGGACT
 1141
 euGluAspArgArgValGluIleThrGlyProValGluArgLysMetValIleAsnAlaL
 TAGAAGACCGCCGCGTAGAGATAACTGGCCCGGTAGAGCGCAAGATGGTGATCAACGCGC
 1201
 euAsnAlaAsnValLysValPheMetAlaAspPheGluAspSerLeuAlaProAspTrpA
 TCAACGCCAATGTGAAAGTCTTTATGGCCGATTTCGAAGATTCACTGGCACCAGACTGGA

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FIG. 6. 2/3

1261
snLysValIleAspGlyGlnIleAsnLeuArgAspAlaValAsnGlyThrIleSerTyrT
ACAAAGTGATCGACGGGCAAATTAACCTGCGTGATGCGGTTAACGGCACCATCAGTTACA
1321
hrAsnGluAlaGlyLysIleTyrGlnLeuLysProAsnProAlaValLeuIleCysArgV
CCAATGAAGCAGGCAAATTTACCAGCTCAAGCCCAATCCAGCGGTTTTGATTTGTCGGG
1381
alArgGlyLeuHisLeuProGluLysHisValThrTrpArgGlyGluAlaIleProGlyS
TACGCGGTCTGCACTTGCCGGAACATGTACCTGGCGTGGTGAGGCAATCCCCGGCA
1441
erLeuPheAspPheAlaLeuTyrPhePheHisAsnTyrGlnAlaLeuLeuAlaLysGlyS
GCCTGTTTGATTTTGCGCTCTATTTCTTCCACAACCTATCAGGCACTGTTGGCAAAGGGCA
1501
erGlyProTyrPheTyrLeuProLysThrGlnSerTrpGlnGluAlaAlaTrpTrpSerG
GTGGTCCCTATTTCTATCTGCCGAAACCCAGTCCTGGCAGGAAGCGGCCTGGTGAGCG
1561
luValPheSerTyrAlaGluAspArgPheAsnLeuProArgGlyThrIleLysAlaThrL
AAGTCTTCAGCTATGCAGAAGATCGCTTTAATCTGCCGCGCGGCACCATCAAGGCGACGT
1621
euLeuIleGluThrLeuProAlaValPheGlnMetAspGluIleLeuHisAlaLeuArgA
TGCTGATTGAAACGCTGCCCGCGGTGTTCCAGATGGATGAAATCCTTCACGCGCTGCGTG
1681
spHisIleValGlyLeuAsnCysGlyArgTrpAspTyrIlePheSerTyrIleLysThrL
ACCATATTGTTGGTCTGAACTGCGGTCGTTGGGATTACATCTTCAGCTATATCAAAACGT
1741
euLysAsnTyrProAspArgValLeuProAspArgGlnAlaValThrMetAspLysProP
TGAAAACTATCCCGATCGCGTCCTGCCAGACAGACAGGCAGTGACGATGGATAAACCAT
1801
heLeuAsnAlaTyrSerArgLeuLeuIleLysThrCysHisLysArgGlyAlaPheAlaM
TCCTGAATGCTTACTCACGCCTGTTGATTAAAACCTGCCATAAACCGCGGTGCTTTTGCGA
1861
etGlyGlyMetAlaAlaPheIleProSerLysAspGluGluHisAsnAsnGlnValLeuA
TGGGCGGCATGGCGGCGTTTATTCCGAGCAAAGATGAAGAGCACATAACCAGGTGCTCA
1921
snLysValLysAlaAspLysSerLeuGluAlaAsnAsnGlyHisAspGlyThrTrpIleA
ACAAAGTAAAAGCGGATAAATCGCTGGAAGCCAATAACGGTCACGATGGCACATGGATCG
1981
laHisProGlyLeuAlaAspThrAlaMetAlaValPheAsnAspIleLeuGlySerArgL
CTCACCCAGGCCTTGCGGACACGGCAATGGCGGTATTCAACGACATTCTCGGCTCCCGTA
2041
ysAsnGlnLeuGluValMetArgGluGlnAspAlaProIleThrAlaAspGlnLeuLeuA
AAAATCAGCTTGAAGTGATGCGCGAACAAGACGCGCCGATTACTGCCGATCAGCTGCTGG
2101
laProCysAspGlyGluArgThrGluGluGlyMetArgAlaAsnIleArgValAlaValG
CACCTTGTGATGGTGAACGCACCGAAGAAGGTATGCGCGCCAACATTGCGGTGGCTGTGC
2161
lnTyrIleGluAlaTrpIleSerGlyAsnGlyCysValProIleTyrGlyLeuMetGluA
AGTACATCGAAGCGTGATCTCTGGCAACGGCTGTGTGCCGATTTATGGCCTGATGGAAG
2221
spAlaAlaThrAlaGluIleSerArgThrSerIleTrpGlnTrpIleHisHisGlnLysT
ATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAA

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FIG. 6 3/3

2281
hrLeuSerAsnGlyLysProValThrLysAlaLeuPheArgGlnMetLeuGlyGluGluM
CGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGTTCCGCCAGATGCTGGGCGAAGAGA
2341
etLysValIleAlaSerGluLeuGlyGluGluArgPheSerGlnGlyArgPheAspAspA
TGAAAGTCATTGCCAGCGAACTGGGCGAAGAACGTTTCTCCCAGGGCGTTTTGACGATG
2401
laAlaArgLeuMetGluGlnIleThrThrSerAspGluLeuIleAspPheLeuThrLeuP
CCGCACGCTTGATGGAACAGATCACCACCTTCCGATGAGTTAATTGATTTCCTGACCCTGC
2461 growth hormone exon 5
roGlyTyrArgLeuLeuAla***
CAGGCTACCGCCTGTTAGCGTAAtttgacctgcgcttcttagttgccagccatctgctgt
2521
taccctcccctgtgccttcctagaccctggaagggtgccactccagtgccaccgctcctt
2581
cttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcattctattctaggg
2641
ggtggggtcgggcaggatagcgagggggaggattgggaagacaatagcaggggtgctgtg
2701
ggctctatgggtacccaggtgctgaataattgacccggttcctcctggggcagaaagaag
2761
caggcacatcccccttctctgtgacacacccggtcctcgcccctggctccttagttccagcc
2821
ccactcataggacactcacagctcaggaggggtccgccttcaatcccaccgcctaaagt
2881
cttgagcggtctctccctctcagccaccagccgaatctaggcctccagagtgggaagaa
2941
tttaagcaagacaggctatgaagtacagaggagagaaaaatgcctccaacatgtgaggaa
3001
gtgatgagagaaagcgtagaattagttttgtggcataaattttaagggtgactacacact
3061
ggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtgtccagctctttg
3121
tgaccccaacggactgtggctgccagggtcctctgtccatgggattctccagggcaagaat
3181
actggagggggttgccattccccaggggatcttcccagcccaaggatcaaaccgagttt
3241
ctgcattgcaggcagattctttactctctgagccatcaggggaagccctgtgggaaatggg
3301
aaccatgcaagaatggctttgggaccaataggaccagaatgtttgggatctgaactgggt
3361
caagagatgtggaagagagattctaaatgcatgtgttcatgctaagtggcttcagtcgtg
3421
tcctactatttgcaaccccgatgaactgcag

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FIG. 7 1/5

SEQUENCE OF THE MTaceAB1 GENE

1 metallothionein promoter
 gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggatgtaattc
 61
 tcaggactattcaaagggaaataccactgtcttacttcgttattggatgccagctctgc
 121
 ccatacattacaaggatgcttttccctagggggccatcctatgactaggggaacctccatcct
 181
 ggagccgggtggactggctagggcagtgattccctggccattcatctattcagtcgtgg
 241
 agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
 301
 aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
 361
 gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
 421
 ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
 481
 gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
 541
 gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaagggggcgggcaggaa
 601
 tcctggagcgctacttgtcattcgggacaaaagtcctccgcgttgggggcgagtaggggg
 661
 acggaggcggttccggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
 721
 cgcgtggtgctcaccgcccagaccgggtgcagcgggcagctcgggtgcaggcgggggag
 781
 accctctgcgcccggccgcctcctgtgggtataatagcgctcggctcctgggctccaac
 841
 bacterial aceA sequence
 MetLysThrArgThrGlnG
 acgcctcccaccggaccagtggatcctctagagtcacaccATGAAAACCGTACACAAC
 901
 lnIleGluGluLeuGlnLysGluTrpThrGlnProArgTrpGluGlyIleThrArgProT
 AAATTGAAGAATTACAGAAAGAGTGGACTCAACCGCGTTGGGAAGGCATTACTCGCCCAT
 961
 yrSerAlaGluAspValValLysLeuArgGlySerValAsnProGluCysThrLeuAlaG
 ACAGTGCGGAAGATGTGGTGAAATTACGCGGTTCACTCAATCCTGAATGCACGCTGGCGC
 1021
 lnLeuGlyAlaAlaLysMetTrpArgLeuLeuHisGlyGluSerLysLysGlyTyrIleA
 AACTGGGCGCAGCGAAAATGTGGCGTCTGCTGCACGGTGAGTCGAAAAAAGGCTACATCA
 1081
 snSerLeuGlyAlaLeuThrGlyGlyGlnAlaLeuGlnGlnAlaLysAlaGlyIleGluA
 ACAGCCTCGGCGCACTGACTGGCGGTCAGGCGCTGCAACAGGCGAAAGCGGGTATTGAAG
 1141
 laValTyrLeuSerGlyTrpGlnValAlaAlaAspAlaAsnLeuAlaAlaSerMetTyrP
 CAGTCTATCTGTCTGGGATGGCAGGTAGCGGCGGACGCTAACCTGGCGGCCAGCATGTATC
 1201
 roAspGlnSerLeuTyrProAlaAsnSerValProAlaValValGluArgIleAsnAsnT
 CGGATCAGTCGCTCTATCCGGCAAACCTCGGTGCCAGCTGTGGTGGAGCGGATCAACAACA

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FIG. 7 2/5

1261
hrPheArgArgAlaAspGlnIleGlnTrpSerAlaGlyIleGluProGlyAspProArgT
CCTTCCGTGCGTGCAGATCCAATGGTCCGCGGCATTGAGCCGGGCGATCCGCGCT
1321
yrValAspTyrPheLeuProIleValAlaAspAlaGluAlaGlyPheGlyGlyValLeuA
ATGTCGATTACTTCCTGCCGATCGTTGCCGATGCGGAAGCCGGTTTTGGCGGTGTCCTGA
1381
snAlaPheGluLeuMetLysAlaMetIleGluAlaGlyAlaAlaAlaValHisPheGluA
ATGCCTTTGAACTGATGAAAGCGATGATTGAAGCCGGTGCAGCGGCAGTTCACCTCGAAG
1441
spGlnLeuAlaSerValLysLysCysGlyHisMetGlyGlyLysValLeuValProThrG
ATCAGCTGGCGTCAGTGAAGAAATGCGGTACATGGGCGGCAAAGTTTTAGTGCCAACTC
1501
lnGluAlaIleGlnLysLeuValAlaAlaArgLeuAlaAlaAspValThrGlyValProT
AGGAAGCTATTTCAGAACTGGTCGCGGCGCGTCTGGCAGCTGACGTGACGGCGTTCCAA
1561
hrLeuLeuValAlaArgThrAspAlaAspAlaAlaAspLeuIleThrSerAspCysAspP
CCCTGCTGGTTGCCCGTACCGATGCTGATGCGGCGGATCTGATCACCTCCGATTGCGACC
1621
roTyrAspSerGluPheIleThrGlyGluArgThrSerGluGlyPhePheArgThrHisA
CGTATGACAGCGAATTTATTACCGCGCAGCGTACCAGTGAAGGCTTCTTCCGTACTCATG
1681
laGlyIleGluGlnAlaIleSerArgGlyLeuAlaTyrAlaProTyrAlaAspLeuValT
CGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCT
1741
rpCysGluThrSerThrProAspLeuGluLeuAlaArgArgPheAlaGlnAlaIleHisA
GGTGTGAAACCTCCACGCCGATCTGGAAGTGGCGCGTCGCTTGCACAAGCTATCCACG
1801
laLysTyrProGlyLysLeuLeuAlaTyrAsnCysSerProSerPheAsnTrpGlnLysA
CGAAATATCCGGGCAAACCTGCTGGCTTATACTGCTCGCCGTCGTTCAACTGGCAGAAAA
1861
snLeuAspAspLysThrIleAlaSerPheGlnGlnGlnLeuSerAspMetGlyTyrLysP
ACCTCGACGACAAAACCTATTGCCAGCTTCCAGCAGCAGCTGTCCGATATGGGCTACAAGT
1921
heGlnPheIleThrLeuAlaGlyIleHisSerMetTrpPheAsnMetPheAspLeuAlaA
TCCAGTTCATCACCTGGCAGGTATCCACAGCATGTGGTTCAACATGTTTGACCTGGCAA
1981
snAlaTyrAlaGlnGlyGluGlyMetLysHisTyrValGluLysValGlnGlnProGluP
ACGCCTATGCCAGGGCGAGGGTATGAAGCACTACGTTGAGAAAGTGCAGCAGCCGGAAT
2041
heAlaAlaAlaLysAspGlyTyrThrPheValSerHisGlnGlnGluValGlyThrGlyT
TTGCCGCCGCGAAAGATGGCTATACCTTCGTATCTCACCAGCAGGAAGTGGGTACAGGTT
2101
yrPheAspLysValThrThrIleIleGlnGlyGlyAspValPheSerHisArgAlaAspA
ACTTCGATAAAGTGACGACTATTATTCAGGGCGGCGACGTCTTCAGTCACCGCGCTGACC
2161 growth hormone exon 5
rgLeuHis***
GGCTCCACTGAagaatcgagttctaatttgacctgcgccttctagttgccagccatctg
2221
ctgttaccctccctgtgccttcctagaccctggaaggtgccactccagtgcccaccgtc
2281
ctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcattctattct

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FIG. 7 3/5

2341
agggggtggggtcgggcaggatagcgagggggaggattgggaagacaatagcaggggtgc
2401
tgtgggctctatgggtacccaggtgctgaataattgaccgggttcctcctggggcagaaa
2461
gaagcaggcacatccccttctctgtgacacacccgggtcctcgcccctggtccttagttcc
2521
agccccactcataggacactcacagctcaggaggggtccgccttcaatcccacccgctaa
2581
agtgccttgagcggtctctccctctcagccaccagccgaatctaggcctccagagtggga
2641
agaatttaagcaagacaggctatgaagtacagagggagagaaaaatgcctccaacatgtga
2701
ggaagtgatgagagaaagcgtagaattagttttgtggcataaatttttaagggtgactacac
2761
acttgcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtgtccagctc
2821
tttgtgaccccaaggactgtgggtgccaggtcctctgtccatgggattctccagggcaa
2881
gaatactggaggggggttgccattccccaggggatcttcccagcccaaggatcaaaccga
2941
gtttctgcattgcaggcagattctttactctctgagccatcaggggaagccctgtgggaaa
3001
tgggaaacctgcaagaatggctttgggaccaataggaccagaatgtttgggatctgaact
3061
gggtcaagagatgtggaagagagattctaaatgcatgtgttcattgctaagtggcttcagt
3121 metallothionein promoter
cgtgtcctactatttgcaaccccgatgaactgcaggaattcaaagaggaaaagtgtgaa
3181
acaaggcttggcacagactccctgggtatgtaattctcaggactattcaaagggaaatacc
3241
cactgtcttacttcgttattggatgccagctctgcccattcacttacaaggatgcttttcc
3301
tagggggcatcctatgactagggaaacctccatcctggagccgggtggactggctaggcag
3361
tggtattccctggcccattcatctattcagtcgtggagaatgtaaggaaggctgggcgaca
3421
gaaggctgagttcgctgctgggctgttacaggagaaaactagagactctgttcaaagtcca
3481
gggtgggggctgtgggaggaaatattaggggaagcgggggttcgggggataggtggtgaagc
3541
tcacatccatcacgggtctctgcacacgacacaggggtccagccaagcctgggatgtga
3601
gcacgaggctcggattgcgcatgagctctgggaaagggtgaaagcaaagacaagagttgc
3661
gggggcagggaagactgcgaggactcagggactgggttcccgtaaacaccgatgactgcc
3721
cacattgtggaagctgggaaggggcgggcaggaatcctggagcgctacttgtcattcgg
3781
gacaaagtcctccgcgttgggggcgagtagggggacggaggcggtttcggtgcgcacgga

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FIG. 7 4/5

3841
gcccagccgcgttccgggaatcttgcgctcggccgcgcgtggtgctcaccgcccgcacccg
3901
ggtgcagcgggcagctcgggtgcaggcgggggcagaccctctgcgcccggcccgcctcct
3961 metallothionein cap site *
gtgggtataatagcgctcggctcctgggctccaacacgcctcccaccggaccagtggatc
4021 bacterial aceB sequence
MetThrGluGlnAlaThrThrThrAspGluLeuAlaPheThrAr
ctctagagtcacccATGACTGAACAGGCAACAACAACCGATGAACTGGCTTTCACAAG
4081
gProTyrGlyGluGlnGluLysGlnIleLeuThrAlaGluAlaValGluPheLeuThrGl
GCCGTATGGCGAGCAGGAGAAGCAAATTCTTACTGCCGAAGCGGTAGAATTTCTGACTGA
4141
uLeuValThrHisPheThrProGlnArgAsnLysLeuLeuAlaAlaArgIleGlnGlnGl
GCTGGTGACGCATTTTACGCCACAACGCAATAAACTTCTGGCAGCGCGCATTCAGCAGCA
4201
nGlnAspIleAspAsnGlyThrLeuProAspPheIleSerGluThrAlaSerIleArgAs
GCAAGATATTGATAACGGAACGTTGCCTGATTTTATTTTCGGAACAGCTTCCATTCGCGA
4261
pAlaAspTrpLysIleArgGlyIleProAlaAspLeuGluAspArgArgValGluIleTh
TGCTGATTGGAATAATTCGCGGGATTCTCTGCGGACTTAGAAGACCGCCGCTAGAGATAAC
4321
rGlyProValGluArgLysMetValIleAsnAlaLeuAsnAlaAsnValLysValPheMe
TGGCCCGGTAGAGCGCAAGATGGTGATCAACGCGCTCAACGCCAATGTGAAAGTCTTTAT
4381
tAlaAspPheGluAspSerLeuAlaProAspTrpAsnLysValIleAspGlyGlnIleAs
GGCCGATTTCTGAAGATTCCTGGCACCAGACTGGAACAAAGTGATCGACGGGCAAATTAA
4441
nLeuArgAspAlaValAsnGlyThrIleSerTyrThrAsnGluAlaGlyLysIleTyrGl
CCTGCGTGATGCGGTTAACGGCACCATCAGTTACACCAATGAAGCAGGCAAATTTACCA
4501
nLeuLysProAsnProAlaValLeuIleCysArgValArgGlyLeuHisLeuProGluLy
GCTCAAGCCCAATCCAGCGGTTTTGATTTGTGCGGTACGCGGTCTGCACTTGCCGGAAAA
4561
sHisValThrTrpArgGlyGluAlaIleProGlySerLeuPheAspPheAlaLeuTyrPh
ACATGTCACCTGGCGTGGTGAGGCAATCCCCGGCAGCCTGTTTGATTTTGCGCTCTATTT
4621
ePheHisAsnTyrGlnAlaLeuLeuAlaLysGlySerGlyProTyrPheTyrLeuProLy
CTTCCACAACATCAGGCACTGTTGGCAAAGGGCAGTGGTCCCTATTTCTATCTGCCGAA
4681
sThrGlnSerTrpGlnGluAlaAlaTrpTrpSerGluValPheSerTyrAlaGluAspAr
AACCCAGTCCTGGCAGGAAGCGGCCTGGTGGAGCGAAGTCTTCAGCTATGCAGAAGATCG
4741
gPheAsnLeuProArgGlyThrIleLysAlaThrLeuLeuIleGluThrLeuProAlaVa
CTTTAATCTGCCGCGCGGCACCATCAAGGCGACGTTGCTGATTGAAACGCTGCCCGCCGT
4801
lPheGlnMetAspGluIleLeuHisAlaLeuArgAspHisIleValGlyLeuAsnCysGl
GTTCCAGATGGATGAAATCCTTCACGCGCTGCGTGACCATATTGTTGGTCTGAACTGCGG
4861
yArgTrpAspTyrIlePheSerTyrIleLysThrLeuLysAsnTyrProAspArgValLe
TCGTTGGGATTACATCTTCAGCTATATCAAAACGTTGAAAACTATCCCGATCGCGTCCT

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FIG. 7 5/5

4921
uProAspArgGlnAlaValThrMetAspLysProPheLeuAsnAlaTyrSerArgLeuLe
GCCAGACAGACAGGCAGTGACGATGGATAAACCATTCCTGAATGCTTACTCACGCCTGTT
4981
uIleLysThrCysHisLysArgGlyAlaPheAlaMetGlyGlyMetAlaAlaPheIlePr
GATTAAACCTGCCATAAACGCGGTGCTTTTTCGATGGGCGGCATGGCGGCGTTTATTCC
5041
oSerLysAspGluGluHisAsnAsnGlnValLeuAsnLysValLysAlaAspLysSerLe
GAGCAAAGATGAAGAGCACATAACCAGGTGCTCAACAAAGTAAAAGCGGATAAATCGCT
5101
uGluAlaAsnAsnGlyHisAspGlyThrTrpIleAlaHisProGlyLeuAlaAspThrAl
GGAAGCCAATAACGGTCACGATGGCACATGGATCGCTCACCCAGGCCTTGCGGACACGGC
5161
aMetAlaValPheAsnAspIleLeuGlySerArgLysAsnGlnLeuGluValMetArgG1
AATGGCGGTATTCAACGACATTCTCGGCTCCCGTAAAAATCAGCTTGAAGTGATGCGCGA
5221
uGlnAspAlaProIleThrAlaAspGlnLeuLeuAlaProCysAspGlyGluArgThrG1
ACAAGACGCGCCGATTACTGCCGATCAGCTGCTGGCACCTTGTGATGGTGAACGCACCGA
5281
uGluGlyMetArgAlaAsnIleArgValAlaValGlnTyrIleGluAlaTrpIleSerG1
AGAAGGTATGCGCGCCAACATTTCGCGTGGCTGTGCAGTACATCGAAGCGTGGATCTCTGG
5341
yAsnGlyCysValProIleTyrGlyLeuMetGluAspAlaAlaThrAlaGluIleSerAr
CAACGGCTGTGTGCCGATTTATGGCCTGATGGAAGATGCGGCGACGGCTGAAATTTCCCG
5401
gThrSerIleTrpGlnTrpIleHisHisGlnLysThrLeuSerAsnGlyLysProValTh
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5461
rLysAlaLeuPheArgGlnMetLeuGlyGluGluMetLysValIleAlaSerGluLeuG1
CAAAGCCTTGTTCCGCCAGATGCTGGGCGAAGAGATGAAAGTCATTGCCAGCGAACTGGG
5521
yGluGluArgPheSerGlnGlyArgPheAspAspAlaAlaArgLeuMetGluGlnIleTh
CGAAGAACGTTTCTCCCAGGGGCGTTTTGACGATGCCGCACGCTTGATGGAACAGATCAC
5581
rThrSerAspGluLeuIleAspPheLeuThrLeuProGlyTyrArgLeuLeuAla***
CACTTCCGATGAGTTAATTGATTTCTGACCCTGCCAGGCTACCGCCTGTTAGCGTAAtt
5641 growth hormone exon 5
tgacctgcgccttctagttgccagccatctgctgttaccctccctgtgccttcctagac
5701
cctggaaggtgccactccagtgcccaccgtcctttcttaataaagcggaggaaattgcat
5761
cacattgtctgagtaggtgtcattctattctagggggtgggggtcgggcaggatagcgagg
5821
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5881
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5941
caccgggtcctcgcccctgggtccttagttccagccccactcataggacactcacagctca

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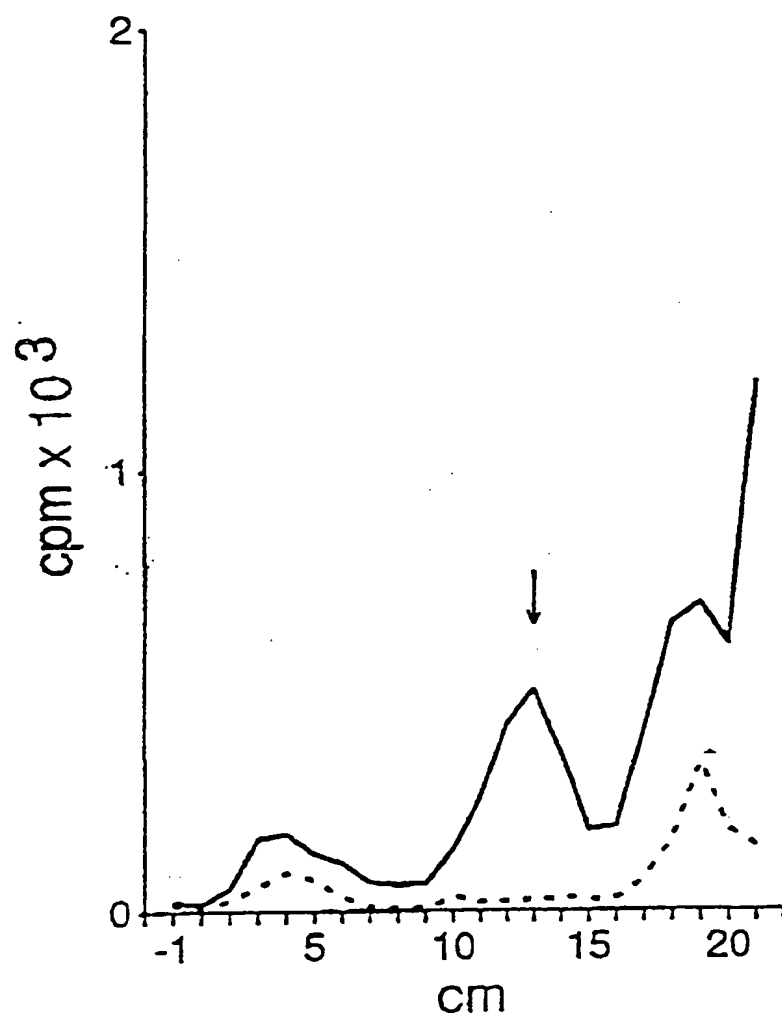


Fig. 8

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)⁶

According to International Patent classification (IPC) or to both National Classification and IPC
Int. Cl.⁶ C12N 15/85, 15/60, 15/67

II. FIELDS SEARCHEDMinimum Documentation Searched⁷

Classification System

Classification Symbols

IPC WPAT Derwent Database: Keywords: inducible, promoter, regulatory, element, exon, non-coding
Chemical Abstracts: Keywords: hormone, exon, non-coding

Documentation Searched other than Minimum Documentation
to the extent that such Documents are included in the Fields Searched⁸

Biotechnology Abstracts: Keywords: growth, hormone, exon, non-coding
AU:IPC:C12N 15/85, 15/60, 15/67, 15/11, 15/18:

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate of the relevant passages ¹²	Relevant to Claim No ¹³
Y	Hampson, R.K. et al. Molecular and Cellular Biology, Volume 9, No. 4, April 1989 (American Society for Microbiology) "Alternative Processing of Bovine Growth Hormone mRNA is Influenced by Downstream Exon Sequences", see pages 1604-1610.	1-7
Y	Byrne, C.R. et al. Australian Journal of Biological Sciences, Volume 40, No. 4, 1987, "The Isolation and Characterisation of the Ovine Growth Hormone Gene", see pages 459-468.	1-7
Y	Orian, J.M. et al. Nucleic Acids Research, Volume 16, No. 18, 1988 (IRL Press Limited) "Cloning and sequencing of the ovine growth hormone gene" see page 9046.	1-7

* Special categories of cited documents : ¹⁰

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

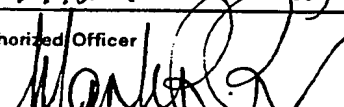
"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 20 June 1992	Date of Mailing of this International Search Report 25 June 1992 (25.06.92)
International Searching Authority AUSTRALIAN PATENT OFFICE	Signature of Authorized Officer M. ROSS 

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A

Curatola, A.M. and C. Basilico.
Molecular and Cellular Biology, Volume 10, N . 6, June 1980
(American Society for Microbiology)
"Expression of the K-fgf Proto-Oncogene Is Controlled by 3¹
Regulatory Elements Which Are Specific for Embryonal Carcinoma
Cells" see pages 2575-2483.

A

Gutkind, J.S. et al. Molecular and Cellular Biology, Volume 11, No. 3,
March 1991 (American Society for Microbiology)
"A Novel c-fgr Exon Utilized in Epstein-Barr Virus-Infected B
Lymphocytes but Not in Normal Monocytes" see pages 1500-1507.

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.